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ORIGINAL PAPER

Biohydrogen production with anaerobic sludge immobilized by granular activated carbon in a continuous stirred-tank

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Abstract: A continuous stirred-tank reactor (CSTR) process with granular activated carbon (GAC) was developed for fermentation hydrogen production from molasses-containing wastewater by mixed microbial cultures. Operation at 35°C, an initial biomass of 17.74 $g \cdot L^{-1}$ and hydraulic retention time (HRT) of 6 h, the CSTR reactor presented a continuous hydrogen production ability of 5.9 $L \cdot d^{-1}$ and the biogas was free of methane throughout the experiment. Dissolved fermentation products were predominated by ethanol and acetate acid, with smaller quantities of propionic acid, butyric acid and valeric acid. It was found that GAC could make the immobilized system durable and stable in response to organic load impacting and low pH value. When the organic loading rate (OLR) ranged from 8 kgCOD/(m^3d) to 4 kgCOD/(m^3d), stable ethanol-type fermentation was formed, and the ethanol and acetate concentrations account for 89% of the total liquid products.

Keywords: hydrogen production; ethanol-type fermentation; CSTR; granular activated carbon; low pH

Introduction

Hydrogen gas is an ideal fuel source and produces no green-house gases, since it generates only water when burned (Das et al. 2001; Levin et al. 2004). Direct and highly efficient conversion of H_2 into electricity by fuel cells makes the application of H_2 energy even more attractive (Benemann 1996). Consequently, low-cost and sufficient supply of H_2 could soon become in urgent demand (Patrick et al. 2000; Kim et al. 2005). Hydrogen can be produced substantially at a high rate by anaerobic fermentation from organic waste or wastewater (Lee et al. 2004; Teplyakow et al. 2002), which has been studied excessively (Jin 1998).

Continuous processes for fermentative H_2 production can be divided into two major categories; namely, suspended systems and immobilized systems (Logan et al. 2002; Kataoka et al. 1997). Suspended systems allow better mass transfer between microorganisms and substrates (Han et al. 2004). However, it is difficult to maintain a sufficient amount of H_2-producing bacterial population in the bioreactor under high hydraulic pressure (or low HRT, hydraulic retention time) because washout of the biomass usually occurs at a low HRT (Gavala et al. 2006). As a result, many research efforts were made on enhancing biomass retention by using physical or biological immobilization approaches (Tao et al. 2007; Ueno et al. 1996).

On the other hand, in many papers, ethanol-type fermentation has been depicted as the most popular pathway for fermentative hydrogen production (Ren et al. 2003). Despite ethanol-type fermentation having many advantages for hydrogen production (Lee et al. 2008; Wu et al. 2003), less research related to ethanol-type fermentation with immobilized system has been carried out.

In the present study, anaerobic sludge immobilized by a granular activated carbon approach was used as the carrier in CSTR (continuous stirred-tank reactor), converting molasses for continuous H_2 production. The CSTR was operated under different substrate concentrations (OLR ranged from 8 kgCOD/m^3d to 24 kgCOD/m^3d) to assess the H_2-producing ability. Continuous H_2 operations were also examined to evaluate the stability and feasibility of using the GAC-immobilized cells in practical H_2-producing processes.

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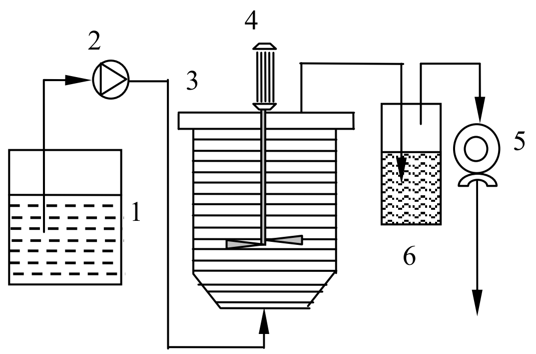
Material and methods

Experimental apparatus and processes

A 12.5L continuous stirred-tank reactor (CSTR) with an effective volume of 5.4 L was used in this study (Fig. 1). The reactor was constructed with transparent plexiglas with a gas-liquid-solid separating device and operated in a continuous flow mode. The temperature was automatically maintained at (35±1)°C by electrothermal wire. The influent flow rate was controlled by a feed pump to regulate the HRT and OLR in the reactor. The biogas generated was collected with a water lock and measured by a wet gas meter (Model LML-1, Changchun Filter Co., Ltd.) which was filled with acidified saturated salt solution to prevent the biogas from dissolution.



1. Waste water box; 2. Feed pump; 3. Reactor; 4. Agitator; 5. Biogas meter; 6. Water lock

Fig. 1 Schematic diagram of the CSTR reactor for biohydrogen production from molasses wastewater

Feeding solution and seed sludge

The molasses used in the experiment were obtained from a local beet sugar refinery (Harbin). Molasses were diluted by tap water to a COD of 10 000 mg/L and the COD: the ratio of nitrogen to phosphorus was maintained at 1000:5:1 by the addition of synthetic fertilizer in the substrate to supply microorganisms with adequate nitrogen and phosphorus contents.

The reactor was inoculated with excess sludge from a secondary settling tank in a local municipal wastewater treatment plant, with a pH of 6.23. It was first sieved through a mesh with a diameter of 0.5 mm in order to remove waste materials that could cause pump failure. The volatile suspended solid (VSS) was 17.74 g·L^{-1}. Hydrogen productivity of the seed sludge was enhanced by aerobition treatment for 30 days to inhibit the methanogenic activity prior to immobilization.

GAC was used as support media for cell immobilization and retention. The particles were sieved for uniformity of approximately 1.5–2 mm in diameter. The main physical characteristics of GAC were offered by supplier as follows: media real density = 1 420 g·L^{-1}, surface area = 1 200–1 350 m^2·g^{-1}, bulk density = 450−500 g·L^{-1} (Hainan Wen chang qiu chi Activated Carbon. Co. Ltd.). H_2-producing sludge was mixed with granular activated carbon at a volume (ml) to weight (g) ratio of 1:10. It was observed that sludge predominantly covered the surface and interior portion of the immobilized disc.

Analytical methods

The biogas yield of the CSTR was measured daily at room temperature using a wet gas meter, and its constituents (H_2 and CO_2) were determined by a gas chromatography (Model GC-122, Shanghai Anal. Inst. Co.). The gas chromatography system was equipped with a thermal conductivity detector and a stainless steel column (2 m×5 mm) filled with Porapak Q (80/100 mesh, Agilent, USA). Nitrogen was used as the carrier gas at the flow rate of 40 mL·min^{-1}.

Volatile fatty acids (VFAs) and ethanol in the fermentation solution were also analyzed by the gas chromatography (Model GC-112, Shanghai Analytical Apparatus Corporation, China) with a hydrogen flame ionization detector and a stainless steel column (2 m×5 mm) packed with a support (GDX103, 60/80 mesh, Shanghai Maikun Chemical Co., Ltd). The operation of the stainless steel column was amenable to temperature programming within 100−200°C. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. Hydrogen was the combustion gas at 50 mL·min^{-1}, and oxygen was the combustion-supporting gas at 500 mL·min^{-1}.

COD, pH and ORP were measured daily in the CSTR.

Results and discussion

Influent COD and COD removal

Fig. 2 and Fig. 3 reflect the variation of OLR and COD removal in the running process, respectively. After one day's operation, COD removal was as high as 66.28%. This is because, on the one hand, the activated sludge has a high metabolic activityon, and on the other hand, it was due to the fact that GAC have a good adsorption property. Immobilized sludge turned from the aerobic acclimation to the anaerobic running lead to metabolic activity of immobilized sludge decreased, and COD removal decreased rapidly to 30% due to GAC absorption saturation. At the same time, the reactor over-acid condition of the system (pH 3.5) severely inhibited the metabolic activity of microbes which lead to COD removal dropped to 18.59% at the fifth day. As the influent COD decreased to 2 000 mg·L^{-1} (OLR 8 kgCOD/m^{3}d), the excessive acid condition of the reactor have been alleviated and microbial metabolic activity gradually restored, which manifested that the COD removal ascent and stabilized at 23%. However, COD removal rapidly increased to 53.98% with organic loading rate (OLR) rose to 24 kgCOD/(m^{3}d) at 13th day. It is common that the increased OLR will trigger a reaction of H_2-producing system, resulting in the decrease of microbial metabolic activity. However, in this test a high COD removal was found when the organic load rate increased to 24

kgCOD/(m^{3}d). The analysis results showed that it was difficult to meet the need of microbial metabolism with the OLR for 8 kgCOD/(m^{3}d); therefore, COD removal rapidly increased rather than decreased after the organic loading increasing to 24 kgCOD/(m^{3}d). On the other hand, in low-load conditions, the microbes digest organic matter adsorbed on the GAC for the metabolism substrate, so that GAC has a certain re-adsorption capacity, which also efficiently contributed to a higher COD removal. However, the system pH value decreased rapidly with a large number of volatile acid productions caused by OLR increasing, leading to the activity of micro-organisms inhibited and COD removal reduced to 11.25%. Although the effluent pH value came up to 4.28 by adding NaOH, COD removal stabilized at 12%, with no significant increase.

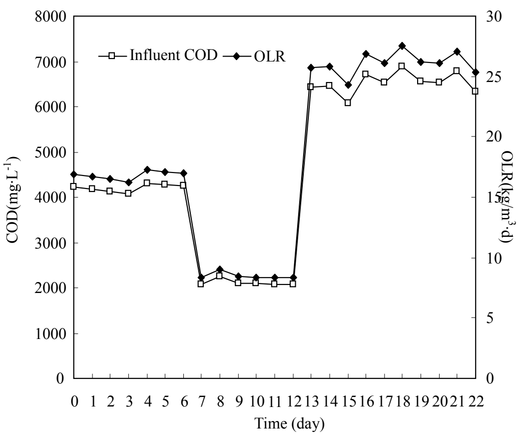


Fig. 2 The variation of OLR during the operation

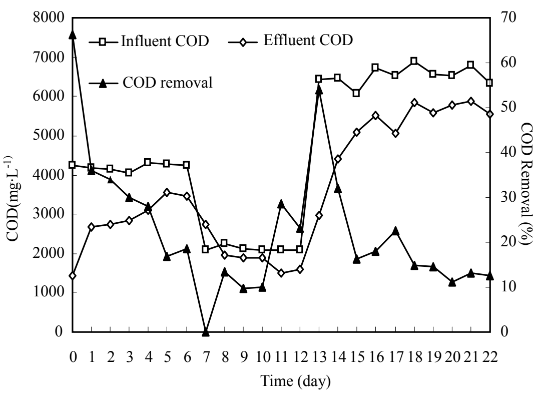


Fig. 3 Influent COD and COD removal variation in immobilized system

Hydrogen production

The biogas and hydrogen yield are generally considered as important indices to evaluate the biohydrogen producing processes. Fig. 4 depicts the biogas and hydrogen yield variations in the fermentation process. The accumulative biogas yield was 25.67 L after three days operation, and reached 4.39 L in the 4th day with the hydrogen yield of 2.27 L. The biogas and hydrogen yield reached 7.7 L·d^{-1} and 3.72 L·d^{-1} after the CSTR system experienced low pH value. However, the OLR decreased from 16 kg COD/(m^{3}d) to 8 kg COD/(m^{3}d) in seven days, the pH value in the system ascended 4 above, and the biogas and hydrogen yield decreased to 5.89 L·d^{-1} and 2.85 L·d^{-1}, respectively.

Compared with the suspended hydrogen production system, the biogas and hydrogen yield did not immediately decrease in the immobilized system because of substrate attached in the granular activated carbon. Although the pH value reached 4.0 and the activity of microbial resumed, the biogas and hydrogen yield decreased after two days because of low OLR. When OLR increased to 24 kg COD/(m^{3}d), the biogas and hydrogen yield increased and reached the max of 11.88 L·d^{-1} and 6.06 L·d^{-1}, respectively. The biogas and hydrogen yield decreased at the 18th day after immobilized system experienced low pH value of 3.4–3.7, which showed that the immobilized system had finite ability of enduring low pH. The biogas was composed of hydrogen and carbon dioxide and free of methane, indicating that the aerobition pretreatment on seed sludge effectively inactivated the methane-forming population.

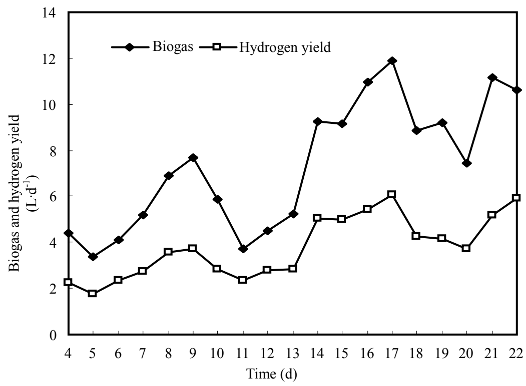


Fig. 4 Biogas and hydrogen yield variation in the immobilized system

Liquid products and ethanol-type fermentation

Fig.5 shows the variation of liquid products during the operation. When the reactor starts, the immobilized system will be in the facultative anaerobic circumstance. The propionic-producing bacterial adapted to facultative anaerobic circumstance and the production of propionic acid was as high as 328.09 mg·L^{-1}. A large number of oxygen molecules attached to the carrier pores in the aerobic biofilm phase and took some time to be consumed by micro-organisms in the anaerobic operation phase. Therefore, propionic acid yield decreased significantly to 191.51 mg·L^{-1} at the 4th day. OLR decreased from 16 kg COD/(m^{3}d) to 8 kg COD/(m^{3}d) in seven days, which lead to the liquid fermentation products dropped to 773.3 mg·L^{-1}. As the H_2-producing bacterial adapted to the anaerobic environment, the immobilized system reached a steady state at 12th day with liquid fermenation products of 1079.68 mg·L^{-1}.

Liquid fermentation products increased from 1 366.14 mg·L^{-1}

to 2 205.58 mg·L^{-1} as OLR raised from 8 kgCOD/(m^{3}d) to 24 kgCOD/(m^{3}d) at the 13th day, which was particularly evident for ethanol content increasing from 542.92 mg·L^{-1} to 791.16 mg·L^{-1}. During the follow operation, the ethanol yield fluctuated between 895.69 mg·L^{-1} and 1095.56 mg·L^{-1} with acetic acid maintained at 764.64–874.77 mg·L^{-1}, while propionic acid, butyric acid and valeric acid were at 18.6–35.46 mg·L^{-1}, 168.86–250.93 mg·L^{-1} and 14.72–26.8 mg·L^{-1} respectively. As the ethanol-type fermentation products, ethanol and acetic acid content accounted for 89% of liquid fermentation products, a typical ethanol-type fermentation formed in the immobilized system.

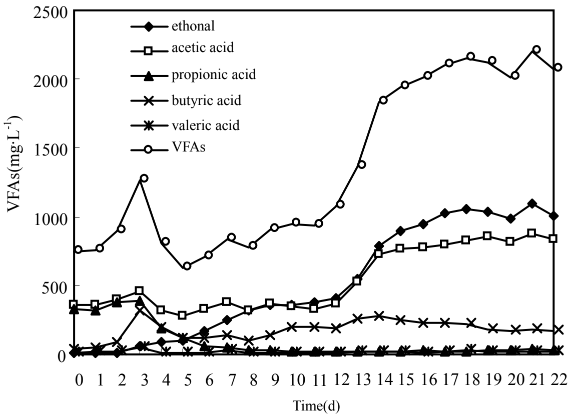


Fig. 5 Liquid metabolic products variation in immobilized system

pH Value

Fig.6 describes the changes of influent pH and effluent pH in the fermentation process. It was observed that influent pH had a small impact on the immobilized system when its value varied from 5.95 to 6.85. On the first day, pH value was not very low (4.92), because micro-organisms could not adapt to the anaerobic environment. However, pH value decreased rapidly to 3.5 in the fifth day due to a large number of volatile acids produced, since the activated sludge gradually adapted to environmental conditions as well as a higher OLR. As OLR dropped from 16 kg/(m^{3}d) to 8 kg/(m^{3}d) in the seventh day, pH value increased gradually and stabilized at 4.14, indicating that it is an effective way to improve the reactor pH by reducing the OLR for immobilized sludge hydrogen fermentation system. However, in 13th day, the pH value dropped to 3.5, which was caused by a large number of volatile acids generated when the organic loading rate raised from 8 kg COD/(m^{3}d) to 24 kgCOD/(m^{3}d). The fact indicated that immobilized system could not improve pH value caused by volatile acid produced. It was also found that the immobilized system could still operate as usual in low pH value, indicating that hydrogen production in immobilized system had a certain ability to resist low pH. When CSTR ran to 20 days, quantitative NaOH was added in influent and pH increased to 4.28 rapidly. Therefore, reducing the organic load, or adding NaOH was a very effective way for improving system pH for immobilized sludge hydrogen fermentation system.

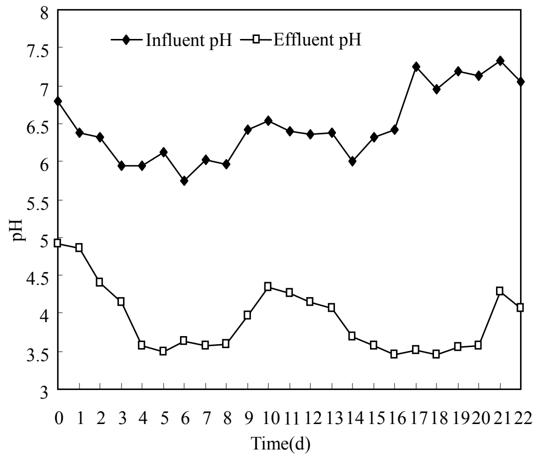


Fig. 6 Influent pH and effluent pH variation in immobilized system

Change of ORP

Oxidation Reduction Potential (ORP) has a significant effect on microbial growth and biochemical metabolism. A variety of intracellular biochemical reactions are oxidation-reduction potential in a particular place. When it is beyond the specified range, the reaction can not occur or the reaction pathway may be changed.

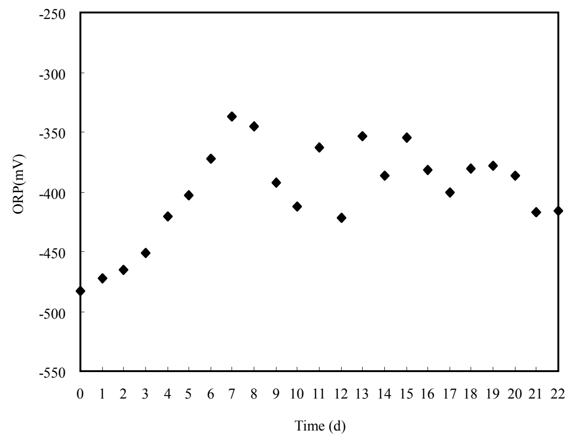


Fig. 7 Variation of ORP in immobilized system

Fig. 7 describes the variation of ORP in immobilized hydrogen fermentation system. After reactor startup, ORP was unstable and had an upward trend (from the beginning of -483 mV up to -337 mV at seventh day). It was because there was certain of oxygen molecules and dissolved oxygen in the immobilized sludge of aeration culture, aerobic biofilm phase and inoculation process, while in the anaerobic phase, the oxygen molecules and dissolved oxygen need to take a period of time to be consumed by microorganisms in the system. In the subsequent operation ORP gradually stabilized at about -420 mV with the formation of anaerobic environment until the establishment of the ethanol-type

fermentation oriented microorganisms. Thus, during the startup process, there is no need of man-made regulation of ORP in the reaction system. As long as the system anaerobic environment can be guaranteed, the reaction system can naturally achieve a lower oxidation-reduction potential by the physiological microbial metabolic activity.

Conclusions

Immobilized sludge biological hydrogen production system with an initial inoculum of 17.74 $g \cdot L^{-1}$, under the conditions, temperature of 35 ± 1°C, HRT for 6 h, OLR at 8–24 kgCOD/(m^3d), could achieve continuous and stable hydrogen production within 22 days. When the ethanol and acetic acid accounted for 89% of the total volatile acid, a typical ethanol-type fermentation would be formed. The biogas and hydrogen yield were 10.6 $L \cdot d^{-1}$ and 5.9 $L \cdot d^{-1}$, respectively.

As the granular activated carbon has a certain absorption capacity, micro-organisms could digest organic matter adsorbed on the GAC as immobilized system running under low OLR. The absorption of GAC makes immobilized system have a certain ability to resist the rapid increase in OLR.

Reducing the organic load rate and adding NaOH are two kinds of effective means of improving the system pH value for immobilized biohydrogen production systems.

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